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PLANT ANIMAL INTERACTIONS

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Dietary specialization influences the efficacy of larval tortoise beetle shield defenses

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Abstract Plant chemical defenses and escape from natural enemies have been postulated to select for dietary specialization in herbivorous insects. In field and laboratory bioassays, we evaluated the effectiveness of intact and chemically modified larval shield defenses of the generalist *Chelymorpha alternans* and the specialists *Acromis sparsa* and *Stolas plagiata* (Chrysomelidae: Cassidinae) against three natural predators, using larvae reared on two morning glory (Convolvulaceae) species. We assessed whether: (1) specialists were better defended than generalists when both were fed and assayed on the same plant; (2) larval shield defenses were chemical, physical, or both; and (3) specialists exploit chemistry better than generalists. Live specialist larvae survived at higher rates than did generalists in predator bioassays with the bug *Montina nigripes* (Reduviidae), but there were no differences among groups against two species of *Azteca* ants (Hymenoptera: Dolichoderinae). Solvent leaching by H_2O or MeOH significantly reduced shield efficacy for all species compared to larvae with intact shields. In contrast, freshly killed specialist larvae exhibited significantly lower capture rates and frequencies than the generalists. Although solvent leaching significantly reduced overall shield efficacy for freshly killed larvae of all species, the pattern of leaching effects differed between specialists and generalists, with H_2O-leaching having a greater impact on the specialists. The overall vulnerability of the generalists appears due to lower chemical protection, which is ameliorated by increased escape behaviors, suggesting a selective trade-off between these defensive components. These experiments indicate that shield defenses are essential for larval survival and that specialists are superior at exploiting plant compounds residing in the aqueous fraction. Our results support the hypothesis that diet-specialized herbivorous insects have more effective defenses than generalists when both feed on the same plant due to the differential ability to exploit defensive precursors obtained from the host. The evolution of dietary specialization may therefore confer the advantage of enhanced enemy-free space.

Keywords Chemical defense · Chrysomelidae · Enemy-free space · Failure-time analysis · Plant/herbivore

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Introduction

Dietary specialization characterizes the vast majority of herbivorous insects (Bernays and Chapman 1994), but the factors responsible for its recurrent evolution are subjects of continued debate (Futuyma and Keese 1992; Thompson 1994; Termonia et al. 2001). The greater risk of failure for a specialized herbivore to locate a suitable host suggests that specialization might have countervailing advantages (Futuyma and Moreno 1988). Ehrlich and Raven (1964) and many later authors postulated that specialists benefit by protecting themselves more effectively against the myriad array of toxic compounds in a few species of plants. The chief competing hypothesis is that specialist herbivores benefit from escaping or more effectively defending themselves against a multitude of natural enemies (Bernays and Graham 1988).

Predators and parasitoids are important sources of herbivore mortality. According to the "enemy-free

space" hypothesis, natural enemies cause differential mortality of an herbivore among potential host species, and the herbivore evolves to feed on only those plants where it suffers lower mortality (Price et al. 1980; Bernays and Graham 1988). Specialization might be favored because a plant provides physical refuge, because a predator does not forage on the plant, or because the plant provides the herbivore with chemicals that the herbivore uses for its own defense. In the latter case, plant chemistry would play a role in the evolution of specialization not because a specialist herbivore may be more resistant to a narrow array of toxins (as postulated by Ehrlich and Raven 1964), but because a specialist may be better able to deploy certain plant compounds for its own defense. That is the hypothesis that our research addresses.

Several studies have described enemy-free space by colonization of rare or novel hosts (Ohsaki and Sato 1990; Keese 1997; Gratton and Welter 1999), by avoidance behaviors (Damman 1987; Bernays 1988; Stamp and Bowers 1992), or by use of physical sanctuaries (Oppenheim and Gould 2002). Many herbivores contain toxic or sub-lethal doses of plant allelochemicals that deter or repel predators (Brower 1984; Dyer and Floyd 1993; Cornelius and Bernays 1995; Dyer 1995; Bowers and Stamp 1997), or that have deleterious post-ingestive effects on predator fitness (Rowell-Rahier and Pasteels 1992). Although this research has revealed a positive relationship between diet breadth and susceptibility to predator attack (Bernays and Graham 1988; Bernays and Cornelius 1989), it is not clear if the advantage of specialization derives from the characteristics of the insect species or from their host plants, since the specialists and generalists in all studies to date were collected or reared from different plant species. For example, among willow-feeding leaf beetles, *Phratora vitellinae* exudes defensive secretions derived from salicylate-rich willows upon which it specializes, whereas *Galerucella lineola* produces no such secretions but uses a wider variety of willow hosts (Denno et al. 1990). Although the specialist is likely better protected, there was no direct assessment of susceptibility to predation for both species on the same host. Despite the generally held opinion that the recurrent evolution of dietary specialization is favored by both adaptation to plant defenses and escape from natural enemies, our understanding of the relative importance of these selective forces and how they interact remains too limited to draw broad evolutionary conclusions (Jaenike 1990; Futuyma and Keese 1992; Stamp 2001).

To investigate the interaction between predation and plant chemistry in relation to herbivore diet range, we examined the larval shield defenses of tortoise beetles. Attached to a highly movable, two-pronged appendage (furca) located at the tip of the abdomen, and held like a parasol above the larva, the shield is a composite structure formed by accumulated feces and molted cuticles. Most species can tilt, aim, and wave their shields rapidly at threats approaching from any direction. Previous studies have demonstrated that shields are not simply physical barriers, but contain a complex chemical component that is necessary for them to function effectively as defenses (Gómez et al. 1999; Vencl et al. 1999; Müller and Hilker 1999). For instance, the shield of the *Solanum*-feeding tortoise beetle *Plagiometriona clavata* is a potent chemical defense based on host-derived compounds (Vencl et al. 1999) and *Cordia*-feeding tortoise beetles incorporate their host's terpenes into their shields to form an effective chemical defense (Gómez et al. 1999). Because these chemical-bearing shields can be experimentally manipulated without harming the larvae, they are well suited for studying the interaction between predation and host chemistry.

We investigated the interaction between diet breadth and predation by measuring the efficacy of intact and chemically modified shield defenses of specialist and generalist larval tortoise beetles against three generalist predators in field and laboratory bioassays. In two diet range contrasts, with larvae reared on the respective specialist's host plant, we subjected the generalist *Chelymorpha alternans* and two specialists, *Acromis sparsa* and *Stolas plagiata* to predation. Specifically, we asked whether: (1) specialists were better defended than generalists when both feed on the same plant; (2) larval shield defenses are chemical, physical, or both; and, (3) specialists defensively exploit chemistry differently than the generalists.

Materials and methods

Tortoise beetles (Coleoptera: Chrysomelidae: Cassidinae) are a widespread, species-rich group of leaf-eating herbivores that reach their highest diversity in the Neotropics. Although a few species feed on monocots, most tortoise beetles (Cassidinae sensu *stricto*) feed on members of five dicotyledonous plant families. Their host plants are common colonists of forest edges, gaps, stream banks, roadsides, and other disturbed areas with high light levels. The entire life cycle occurs on the host. Larvae are totally exposed as they feed on leaf surfaces, and suffer nearly 65% of total life-cycle mortality, inflicted principally by invertebrate generalist predators such as bugs, ants and wasps (Cox 1996; Olmstead 1996). Of the more than 100 species with host information, the vast majority of diets lie well toward the specialized end of the diet spectrum, typically feeding on one member of a single plant genus (Buzzi 1988; Windsor et al. 1992).

We compared three tortoise beetle species that feed on vines in the morning glory family Convolvulaceae (Buzzi 1988; Windsor et al. 1992). Diet contrast 1 compared *A. sparsa* Boheman, a specialist on *Merremia umbellata*, against the generalist, *C. alternans* Boheman, which feeds on at least seven species in two genera (Jansegers 2004), but was reared on *M. umbellata* (hereafter referred to as *CM*). Diet contrast 2 consisted of *S. plagiata* Boheman, a specialist on *Ipomoea phillo-*

mega, and the generalist *C. alternans*, also reared on *I. phillomega* (hereafter referred to as *CI*). Each contrast was subjected to bioassays using a reduviid bug, *Montina nigripes* Stål (Reduviidae), in the laboratory, and two *Azteca* ant species, *A. lacrymosa* Forel and *A. chartifex* Forel (Hymenoptera: Formicidae: Dolichoderinae), in the field. All bioassay experiments were conducted in Gamboa, Republic of Panamá during May through October of 2002 and 2003.

Husbandry

To provide an adequate supply of undamaged beetle fodder, cuttings were made from five field-collected individuals of each of the two host plants and propagated in soil-filled 10-l plastic pots. The growing facility, located at the Gamboa Rainforest Resort, was covered by shade cloth that reduced incident light by 30%, and was equipped with a sprinkler system. Plants were fertilized every 2 weeks.

To assure adequate supplies of parasitoid-free larvae for bioassays, we established stock populations of beetles. Adult beetles were field-collected before the onset of the rainy season (May–April). Located in the Tupper Laboratory of the Smithsonian Tropical Research Institute, Panamá, stocks were maintained at 27°C and a photoperiod of L14:D10. Thirty replicate groups of several males and a female of each species were maintained separately in 473-ml plastic food containers with clear lids, plastic mesh for aeration, and moistened filter paper. Each cup was supplied with one fresh, intact host leaf for every 2 days. Egg masses, averaging 25–35 eggs, were collected daily. Larvae were maintained in food containers supplied with host leaves.

Field-collected *M. nigripes* bugs were individually maintained in 473-ml plastic food containers with a clear lid and a moist cotton ball. Bugs were maintained under the laboratory conditions as described above, and were fed every third day with one or two nymphal grasshoppers or sting-less bees (*Trigona flaviventris*). Once a week, each bug received a cotton ball soaked with dissolved honey.

Bug bioassay with live larvae

We used the generalist predator bug *M. nigripes* in a bioassay to examine if the susceptibilities of specialists and generalists differed against a larval predator suspected to be capable of circumventing shield defenses with its long beak. A bug experiment consisted of two 20-min assays separated by at least 1 h. In the first 20-min assay, test larvae were randomly selected from one species in a contrast and were presented singly to each bug in a plastic cup that served as a test arena. In the second assay, a larva from the corresponding species within a contrast was presented to each bug. Only one experiment was done on a given morning. Bioassays were conducted under the previously described laboratory conditions. One hour prior to the experiment's start, a leaf of the appropriate host contrast was placed in each bug's cup. The presentation protocol consisted of the introduction of a single, shielded fourth instar larva to each bug. The larva was placed on the host leaf at the bottom of the cup at the furthest distance possible (≥7.5 cm) from the bug. As soon as a bug made contact with the prothoracic legs and began beak extension, the test larva was removed. Capture latency—the elapsed time of first orientation to the attack/kill phase of bug prey capture—was measured using a digital stopwatch. We used eight *M. nigripes* bugs in five two-part assay experiments. In all, 40 larvae representing each species were presented. Latency data for the first 5 min of each experiment were pooled and analyzed using failure-time analyses described below.

Ant bioassay with live larvae

Azteca ant bioassays were conducted in the field between 8:00 a.m. and 12:00 noon, from July through September of 2002 and 2003. *Azteca* ants are ubiquitous generalist predators in lowland rainforests (Carroll 1983; Hölldobler and Wilson 1990). *Azteca lacrymosa*, which builds nests attached to the boles of trees, is a very aggressive and strongly recruiting species. *Azteca chartifex* is smaller, builds pendant carton nests, and is less strongly recruiting. Voucher specimens of workers of both species are deposited in the insect collection of the Smithsonian Tropical Research Institute, Panamá. One month before the bioassay experiments began, we set potted individuals of the host plants, *I. phillomega* and of *M. umbellata* (Convolvulaceae), at the bases of two trees with *Azteca* nests. Host vines were placed in contact with the tree trunk to enable the ants to use them as foraging areas.

A bioassay trial consisted of the presentation of an individual larva on its host plant to foraging ants. Each trial was performed on a different host leaf that formed the bioassay test arena. An assay trial was conducted if there were two to five ants foraging on a leaf. Using soft forceps, we placed an experimental larva on a host plant leaf near its center and along the mid-vein. Individual trials were separated from one another by 2–3 min. Sets of ten trials were separated by a 20-min break. These delays served to minimize recruitment interactions across the plant during the course of each morning's experiments. If less than three ants contacted the test larva within each minute, the trial was not scored. Depending on which occurred first, each bioassay trial lasted 5 min or until a larva was captured. A larva was considered captured when the ants carried it ≥1 cm toward the leaf petiole.

Each bioassay trial was recorded using a Panasonic digital video camera (PV-DV951) mounted on a tripod positioned above the test leaf so as to include the entire leaf. We started video recording at the first contact of an

ant with the larva. If a larva moved to the underside or fell off the leaf, the trial was discontinued. We analyzed each bioassay videotape frame-by-frame and measured larval capture time (the interval from first ant contact to time of capture), the distance a larva crawled after first ant contact, and the frequency of shield waving. Distance was calibrated to ant body length measured from head to the tip of the abdomen (*A. lacrymosa* major body length mean ± SE: 5.23 ± 0.05 mm, $n = 15$; *A. chartifex* major body length mean ± SE: 3.07 ± 0.07 mm, $n = 15$). Calipers were adjusted to the appropriate ant size and the distance a larva crawled was measured from the television screen.

Ant bioassay with live larvae with manipulated shields

Shields of live larvae from each species were subjected to manipulation and two leaching treatments. As external structures, shields were manipulated by procedures that did not affect their maneuverability or harm the larvae (Olmstead and Denno 1993; Vencl et al. 1999). Fourth-instar larval sibships of each species–host plant combination were equally divided at random among the following treatment groups: (1) water (H_2O); (2) methanol (MeOH); and (3) unleached (intact) control. We removed shields by placing fine forceps between the tines of the furca and gently lifting the shield away from the body. Solvent-treated shields were soaked for 25–30 min in a solvent bath agitated every 5 min. Shields were then dried on paper toweling under an incandescent light bulb and slow fan for 45 min before reattachment. In the meantime, the larvae were washed in water to remove any residual fecal matter. Each shield was reattached to the larval furca using a rapid-setting, fumeless, water-insoluble craft glue (DAP), and allowed to dry at least 20 min before bioassays were begun. Controls consisted of only the shield removal and reattachment manipulations over the same time intervals. Individual trials were conducted and scored as described above.

Ant bioassay with freshly killed larvae

To eliminate behavior, larvae were frozen (−2°C) 10 min prior to shield manipulation. Shields of fourth instar larvae were either intact or modified using solvents and then presented to the *Azteca* ants by procedures described above. A bioassay trial consisted of the presentation of an individual larva on its host plant to foraging ants, using the protocol described above. Capture time, defined above, was measured using a digital stopwatch.

Statistical analyses

Predation patterns were qualitatively similar for both *Azteca* species, so we combined data to improve our power to detect differences among samples. We examined larval capture times using failure-time statistics (PROC LIFETEST; SAS 2004). In contrast to classical methods such as ANOVA that compare either the total number of captures at the end of the experimental time interval or the average capture time among treatment groups, failure-time methods compare the distributions of capture times throughout the entire bioassay period (Kalbfleisch and Prentice 1980; Fox 2001). Times to the occurrence of an event (e.g., capture of a larva by ants) do not typically meet the distributional assumptions required by traditional parametric approaches. In addition, many of the trials ended before a capture event was recorded (i.e., right-censored data) and the ultimate fate of the larva beyond the bioassay interval was unknown. Capture functions were compared using the Wilcoxon's signed ranks test followed by pairwise multiple comparisons to determine specific differences between treatment groups (Kalbfleisch and Prentice 1980). Significance levels were corrected with the sequential Bonferroni technique (Dunn-Sidák method; Sokal and Rohlf 1995). This method is less conservative than the standard Bonferroni technique but ensures that an appropriate experiment-wise error rate ($\alpha = 0.05$) is maintained. Capture frequency data were analyzed with *G*-tests of independence (Sokal and Rohlf 1995).

Results

Live larvae with intact shields

In both diet contrasts, bugs took significantly longer to capture specialists with intact, unleached shields than generalists (Fig. 1a, b). A similar pattern was seen when the frequency of capture was evaluated, although the difference between specialist and generalist was only significant in diet 2 (Fig. 2a) In contrast, results of the *Azteca* ant bioassay using live larvae with intact shields revealed that the generalist, *C. alternans*, fared as well as the contrasting specialists in both comparisons (Fig. 1c, d). Similarly, the proportion of larvae that survived was uniformly high for the unleached, intact controls in the ant bioassay (Fig. 2b).

Behavior of larvae

Regardless of diet range, larvae reacted to ant attack by rapid crawling and by shield-waving. However, the generalists attempted to flee significantly more vigorously than their specialist counterparts in both contrasts (Table 1). In diet contrast 1, generalist *CM* larvae also waved their shields significantly more frequently than did specialist *A. sparsa* larvae; however, there was no significant difference in the mean frequency of shield waving between the specialist and generalist in diet contrast 2 (Table 1).

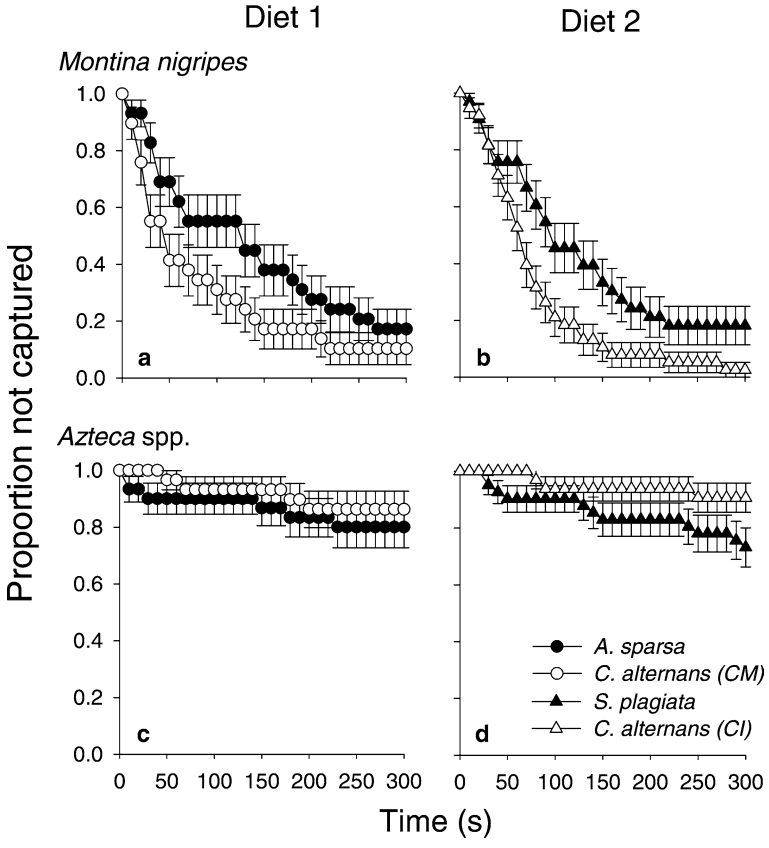

Fig. 1 Proportion (±SE) of uncaptured live specialist [(*A. sparsa* (*filled circle*) or *S. plagiata* (*filled triangle*)] and generalist [(*C. alternans*: *CM* (*open circle*) and *CI* (*open triangle*)] tortoise beetle larvae with unleached, intact shields reared from two diets (*Diet 1 M. umbellata* or *Diet 2 Ipomoaea phillomega*) after exposure to two predators (*M. nigripes* or *Azteca spp.*). Specialists represented by *solid* and generalists by *open symbols*. Larvae were assessed as uncaptured after 5 min in the *Montina* and *Azteca* assays. In the *Montina* assay, $n = 29$ (*A. sparsa* and *CM*), 33 (*S. plagiata*), and 38 (*CI*). In the *Azteca* assay, $n = 30$ (*A. sparsa*), 29 (*CM*), 41 (*S. plagiata*), and 32 (*CI*). *P* values are from Wilcoxon's signed ranks tests: (**a**) 0.035; (**b**) 0.019; (**c**) 0.487; (**d**) 0.067

Live larvae with manipulated shields

The picture of larval resistance changed when their shields were subjected to solvent leaching. Overall, leaching with H_2O significantly increased the vulnerability of all live larvae regardless of diet range (Fig. 3; Table 2). MeOH leaching significantly increased the susceptibility of both generalists to capture but had much less effect on the specialists (Fig. 3; Table 2). It should be noted, however, that the MeOH curves for both specialists appear similar to those for the generalist, suggesting that the failure to detect a difference may be more due to low statistical power than to a lack of effect (see also the results for freshly killed larvae below). Regardless of diet range, larvae were captured significantly more frequently after their shields were modified by solvent leaching compared to unleached controls in the ant bioassay (Fig. 4). However, there were no significant

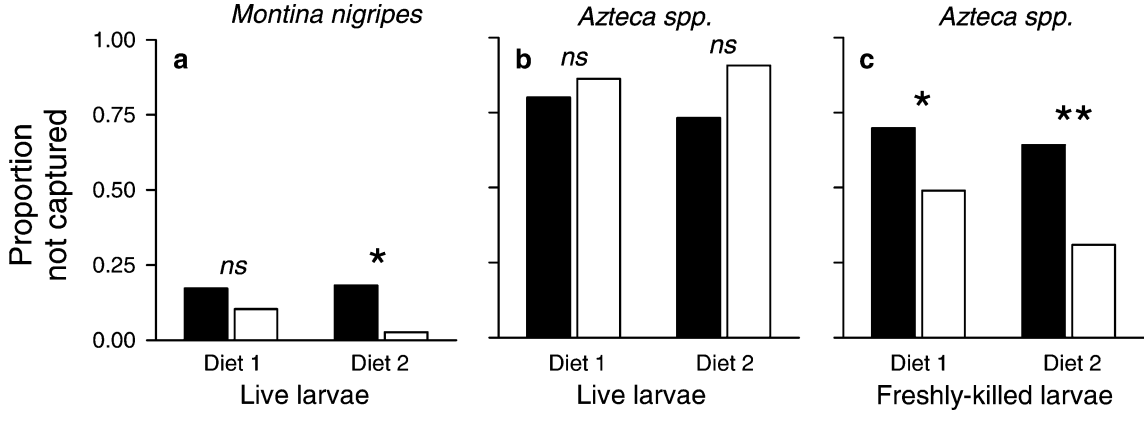

Fig. 2 Proportion of uncaptured live or freshly killed specialist [*A. sparsa* or *S. plagiata* (*solid bars*)] and generalist [*C. alternans*: *CM* and *CI* (*open bars*)] larvae with unleached intact shields reared from two diets (see Fig. 1) after exposure to **a** *M. nigripes* or **b**, **c** *Azteca spp.* predators. Sample sizes are given in Fig. 1 (live larvae) and Fig. 5 (freshly killed larvae). Significance of *G*-tests comparing survival frequency within each diet are shown above the relevant bars for each species (*$P < 0.05$; **$P < 0.01$)

Table 1 Behavioral comparison of specialist and generalist larval tortoise beetles representing two diet contrasts with intact (unleached) shields during the *Azteca* ant bioassays

Diet contrast/beetle species	Larval movement (mm)[a]	P[b]	Shield-waving[c]	P[b]	n[d]
Contrast 1					
A. sparsa	2.98 ± 0.76	< 0.001	2.88 ± 0.61	< 0.01	30
CM	21.95 ± 3.88		6.48 ± 0.99		31
Contrast 2					
S. plagiata	17.56 ± 3.84	< 0.05	7.84 ± 0.70	> 0.05	43
CI	30.41 ± 3.95		7.03 ± 0.80		33

[a]Movement (mean ± SE) of larvae in first minute of each trial
[b]Results of Mann–Whitney *U*-test between specialist and generalist within a contrast
[c]Frequency (mean ± SE) of shield flicks in the first minute of each trial
[d]Number of replicate trials with both *Azteca* species

differences in capture frequencies between MeOH- and H_2O-leaching treatments among specialists and generalists in either diet contrast, or between generalists feeding on different host plants (*G*-tests; all $P > 0.05$).

Freshly killed larvae with intact shields

In the absence of larval behavior such as fleeing or shield-waving, specialist larvae of both diets with intact shields remained uncaptured significantly longer than the respective generalist fed on the same host (Fig. 5a, b). Moreover, in both diet contrasts, a significantly higher proportion of the specialist larvae remained uncaptured during the 5 min bioassay period (Fig. 2c).

Resistance of freshly killed larvae with solvent-leached shields

Results of the bioassays with live larvae suggested that chemistry is an important component of shield effectiveness. This hypothesis was further supported when we presented ants with freshly killed larvae whose shields had been treated with different solvents. The time to capture by ants for larvae whose shields were treated with either MeOH or H_2O was significantly shorter compared to their respective intact controls in all species (Fig. 6; Table 3). Similarly, there were statistically significant reductions in the frequency of larval capture between intact controls and both leaching treatments in all species (Fig. 4).

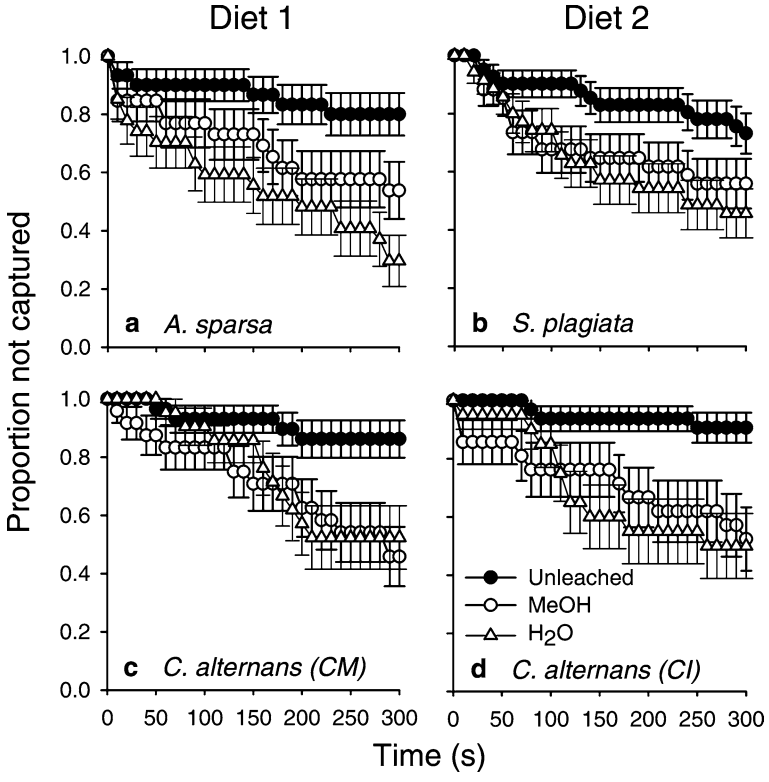

Fig. 3 Proportion (±SE) of uncaptured live specialists (**a** *A. sparsa*, **b** *S. plagiata*) and generalists (*C. alternans*: **c** *CM*; **d** *CI*) larvae with unleached, intact (*filled circle*), MeOH-leached (*open circle*), or H_2O-leached (*open triangle*) shields reared from two diets (see Fig. 1) after exposure to *Azteca* ant predators. For **a** *A. sparsa*, $n = 30$, 26, and 27; for **b** *S. plagiata* $n = 41$, 34, and 35; for **c** *CM* $n = 29$, 24, and 21; and for **d** *CI* $n = 32$, 21 and 20 for the intact, MeOH, and H_2O treatments respectively. *P* values are from Wilcoxon's signed ranks tests: (**a**) 0.003; (**b**) 0.036; (**c**) 0.011; (**d**) 0.002. Statistical results of Bonferroni-corrected pairwise comparisons are presented in Table 2

Table 2 Pairwise multiple comparisons of capture curves for live specialist (*A. sparsa* and *S. plagiata*) and generalist (*C. alternans*) tortoise beetle larvae with intact and solvent-leached shields in *Azteca* ant bioassay (Fig. 3)

Shield treatment	Diet 1		Diet 2	
	A. sparsa	*CM*	*S. plagiata*	*CI*
Intact vs MeOH	NS	*	NS	*
Intact vs H_2O	*	*	*	*
MeOH vs H_2O	NS	NS	NS	NS

Larvae of both diet ranges were raised on either *M. umbellata* (Diet 1) or *Ipomoea phillomega* (Diet 2). To keep the experiment-wise error rate at the 0.05 level, comparisons were done using a sequential Bonferroni approach (Dunn-Sidák method; Sokal and Rohlf 1995) following Wilcoxon's signed ranks tests. Individual comparisons marked with an asterisk (*) were statistically significant at the experiment-wise error rate

In addition, there were important qualitative differences in the effects of shield leaching on larval resistance within both diet contrasts. In contrast 1, capture rates for specialist larvae with H_2O-leached shields were significantly higher than for individuals in the MeOH treatment. Conversely, capture rates for generalist larvae with H_2O-leached shields were significantly lower than for those with MeOH-leached shields (Fig. 6; Table 3). Direct comparison of the curves for the two species (*A. sparsa* and *CM*) showed that although the capture rates of specialist and generalist larvae with MeOH-leached shields were significantly different, the same was not true for those in the H_2O treatment (Fig. 6; Table 4). As in contrast 1, capture rates for specialist larvae with H_2O-leached shields in diet 2 were significantly higher than for individuals in the MeOH treatment; however, while the overall pattern of treatment effects on generalist larvae in diet 2 was similar to that found in diet 1, capture rates for generalists with H_2O- and MeOH-leached shields did not differ statistically (Fig. 6; Table 3). Nevertheless, as in diet 1 the capture curves of the specialist and generalist larvae (*S. plagiata* and *CI*, respectively) were significantly different in the MeOH but not in the H_2O treatment groups (Fig. 6; Table 4).

When we ranked shield treatment effects according to decreasing resistance to predation, the order of effectiveness for the specialists in both diet contrasts was: intact > MeOH > H_2O. However, the positions of MeOH and H_2O were reversed in the rank order for the generalists. For *CM* the rank order was: intact > H_2O > MeOH; and the order for *CI* was: intact > $H_2O \geq$ MeOH (Fig. 6; Table 3).

Discussion

Are specialists better defended than generalists?

Overall, our findings demonstrate that against three of the most common generalist predators in tortoise beetle habitat, these specialists held an advantage over their generalist counterparts when both were reared, and assayed, on the same host plant. Live specialist larvae with unleached shields outlasted the generalist in the bug

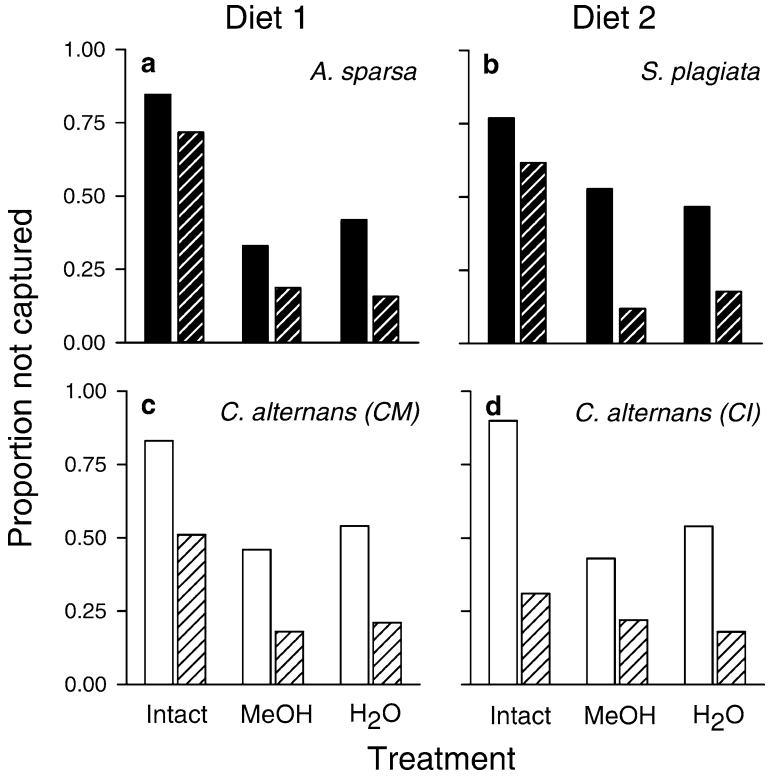

Fig. 4 Proportion of uncaptured live (*solid bars*) and freshly killed (*cross-hatched bars*) tortoise beetle larvae with intact and solvent-leached (MeOH or H_2O) shields after exposure to *Azteca* ant predators for diet 1 (**a**, **c**) and diet 2 (**b**, **d**). Specialists are shown with *black bars*; generalists with *white bars*. Sample sizes are given in Figs. 3 (live larvae) and 6 (freshly killed larvae). Comparisons using *G*-tests of capture frequency between intact controls and leaching treatments within each species for both live and freshly killed larvae were all significant at $P \leq 0.05$

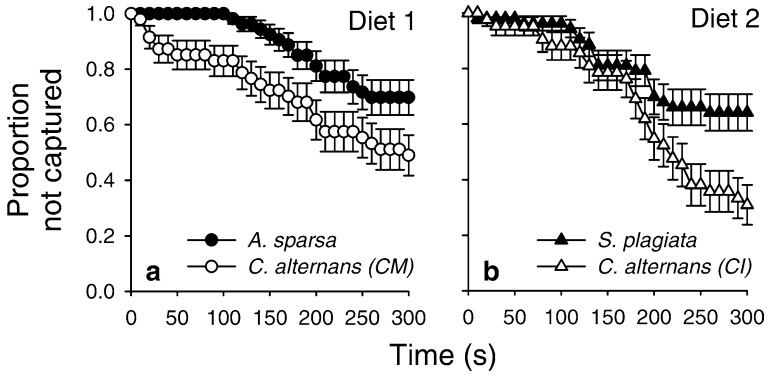

Fig. 5 Proportion (±SE) of uncaptured freshly killed specialist and generalist larvae with intact shields in **a** diet contrast 1 ($P = 0.011$) and in **b** diet contrast 2 ($P = 0.012$) after attack by *Azteca* ants. The specialists *A. sparsa* ($n = 53$) and *S. plagiata* ($n = 53$) are represented by *solid symbols* and the generalist, *C. alternans*, [(*CM* ($n = 47$); *CI* ($n = 42$)] by *open symbols*. *P* values are from Wilcoxon's signed ranks tests

bioassays. Specialist and generalist beetle larvae possessing mobile, unmodified shields and the ability to flee, were equally well protected against *Azteca* ants. However, after avoidance behaviors were eliminated, both specialists were significantly less susceptible to ant attack than the generalists.

Some predators can inflict high mortality on tortoise beetle larvae, especially under experimental conditions (Olmstead 1996). For example, tortoise beetle shields have been shown to be ineffective at thwarting bug attacks because heteropterans can insert their long rostra through or under shields (Olmstead and Denno 1993; Müller 2002). Although generalist predators are considered important determiners of diet breadth (Price et al. 1980; Bernays and Graham 1988), our results might have been different if we had used predators that specialize on tortoise beetle larvae (e.g., Gross et al. 2004).

Since almost all larvae were attacked in our laboratory bug bioassay, shields initially appeared to be ineffective. However, failure-time analyses revealed that specialists enjoyed significantly lower capture rates, even in a brief 5-min bioassay. According to optimal foraging theory, resistance characters that increase the time required to subdue, handle, and assimilate prey may induce opportunistic generalist predators, like bugs, to switch to different, less costly prey that can be more efficiently dispatched (Rabb and Lawson 1957; Pyke 1984; Paradise and Stamp 1990; Olmstead and Denno 1993). Furthermore, some invertebrate predators can learn to avoid unpalatable prey and so may switch to more palatable prey before further direct encounters

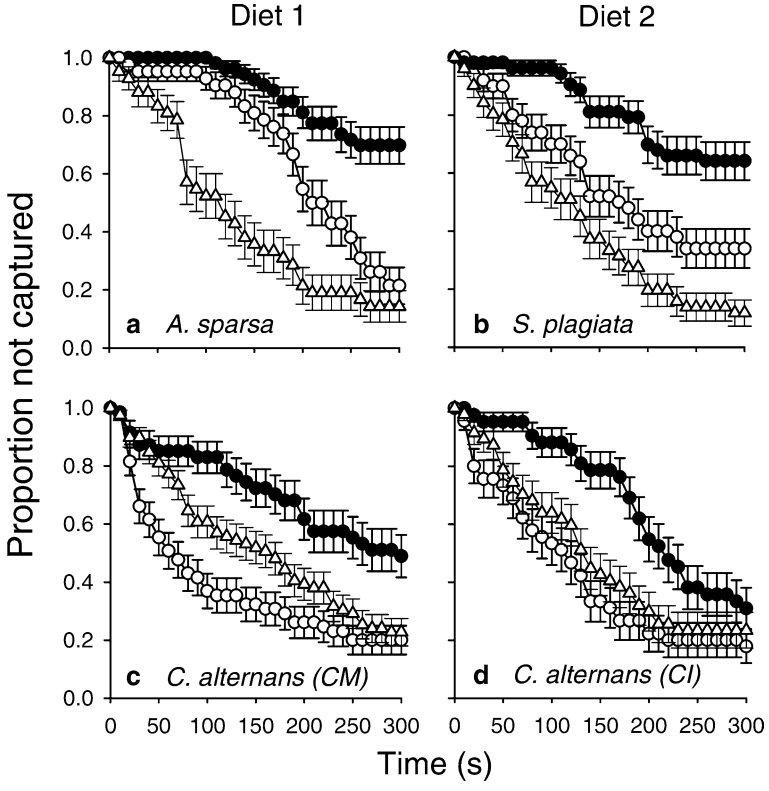

Fig. 6 Proportion (±SE) of uncaptured larvae of freshly killed specialists **a** *A. sparsa* and **b** *S. plagiata* and the generalist *C. alternans* (**c** *CM*; **d** *CI*) with intact (*filled circle*), MeOH-leached (*open circle*), or H_2O-leached (*open triangle*) shields reared on two diets after attack by *Azteca* ants. For **a** *A. sparsa*, $n = 53$, 47, and 42; for **b** *S. plagiata*, $n = 53$, 50, and 51; for **c** *CM*, $n = 47$, 65, and 79; and for **d** *CI*, $n = 42$, 45, and 47 for the intact, MeOH and H_2O treatments, respectively. *P* values from Wilcoxon's signed ranks tests were < 0.001 in all panels. Statistical results of Bonferroni-corrected pairwise comparisons are presented in Table 3

Table 3 Pairwise multiple comparisons of capture curves for freshly killed specialist (*A. sparsa* and *S. plagiata*) and generalist (*C. alternans*) tortoise beetle larvae with intact and solvent-leached shields in the *Azteca* ant bioassay (Fig. 6)

Shield treatment	Diet 1		Diet 2	
	A. sparsa	*CM*	*S. plagiata*	*CI*
Intact vs MeOH	*	*	*	*
Intact vs H_2O	*	*	*	*
MeOH vs H_2O	*	*	*	NS

Larvae of both diet ranges were raised on either *M. umbellata* (Diet 1) or *Ipomoea phillomega* (Diet 2). Comparisons were done using a sequential Bonferroni approach (0.05 level; Dunn-Sidák method; Sokal and Rohlf 1995) following Wilcoxon's signed ranks tests. Individual comparisons marked with an asterisk (*) were statistically significant

Table 4 Pairwise multiple comparisons of cumulative capture curves for freshly killed specialist (*A. sparsa* and *S. plagiata*) and generalist (*C. alternans*) tortoise beetle larvae with intact and solvent-leached shields in the *Azteca* ant bioassay (Fig. 6)

Diet contrast/species	Intact	MeOH	H_2O
Diet 1			
A. sparsa vs *CM*	*	*	NS
Diet 2			
S. plagiata vs *CI*	*	*	NS

Larvae of both diet ranges were raised on either *M. umbellata* (Diet 1) or *Ipomoea phillomega* (Diet 2). Comparisons were done using a sequential Bonferroni approach (0.05 level; Dunn-Sidák method; Sokal and Rohlf 1995) following Wilcoxen signed-rank tests. Individual comparisons marked with an asterisk (*) were statistically significant

(Bernays 1989; Paradise and Stamp 1991). Specialist beetle larvae may have enjoyed lower capture rates by experimental bugs because of their escape behaviors, shield chemistry, or both.

There is scant evidence that specialists gain enemy-free space by incorporating host-derived compounds into predator defenses or by other means that increase their resistance to attack (Berdegue et al. 1996; Stamp 2001). Several studies showed reduced vulnerability of specialist caterpillars reared on or collected from different host plants (Bernays 1988, 1989; Bernays and Cornelius 1989; Dyer and Floyd 1993). Extracts of specialist caterpillars were found to be more deterrent than those of generalists, but again these extracts came from caterpillars with different hosts (Dyer and Floyd 1993; Dyer 1995, 1997). In two studies comparing caterpillars reared on the same plant, the specialist caterpillar was actually more vulnerable (Stamp 1992) and no relationship was found between host concentration of sequesterable compounds and host use by generalist or specialists in the presence of predators (Stamp and Bowers 2000). In the *Phratora-Galerucella* study cited previously, Denno et al. (1990) compared a specialist and generalist on the same host, but did not directly asses their vulnerabilities to predation.

Live specialist and generalist larvae in our study appeared equally resistant to ant predation. Compared to the specialists, generalist larvae responded to ant attack with increased defensive behaviors (escape or shield-waving). The ability to flee quickly and to reduce their apparancy by hiding is an important defense of many species of diurnal, surface-grazing generalist caterpillars (Bernays 1988; Stamp 1992; Stamp and Bowers 1992). However, the susceptibility to ant attack of generalist larvae, deprived of behavioral tactics by having been freshly killed, was significantly higher. The similar resistances of specialists and generalists thus appears due to a combination of the lower chemical and the higher behavioral defenses, reflecting a possible trade-off between these two components in the generalist.

If behavioral defenses are more costly than chemical ones, specialists may gain an advantage by allocating fewer resources to them. Several studies have reported costs associated with avoidance behaviors such as reduced feeding time that can result in negative fitness consequences in invertebrate herbivores ((Lima 1998; Stamp 1992; DeWitt et al. 2000). Evidence of negative correlations between defensive characters is scarce, but trade-offs were found between life history and morphological traits (Rundle and Brönmark 2001). One study measuring the performance of shielded and unshielded tortoise beetle larvae failed to detect any cost per se for shield possession (Olmstead and Denno 1992). The relative costs of chemical and behavioral defenses are not currently known.

Is the shield defense chemical, physical, or both?

Shields of species lacking fecula (feces), and presumably chemical components therein, function as simple physical barriers that thwart attacking enemies (Root and Messina 1983; Eisner and Eisner 2000). Tortoise beetles that vigorously wave their shields can beat back attacking predators (Eisner et al. 1967; Gómez 1997; Chaboo 2002; Müller 2002; Nogueira-de-Sá and Trigo 2002); however, shields appear to be more than simple barriers. Chemical factors contribute to effective shield function in all our study species. When the chemical component is modified in the shields of both live and freshly killed larvae that lack behavior, shield effectiveness against ants is reduced and larvae become significantly more vulnerable to predation.

Do specialists and generalists exploit host chemistry differently?

The strongest evidence that specialists utilize their host plants more effectively to mount a stronger defense comes from the MeOH-leaching treatments. MeOH-leaching significantly reduces resistance to the capture of the generalists more so than the specialists, suggesting that the shields of the specialists rely more heavily upon

compounds in the aqueous fraction (remaining after MeOH-leaching) than do their generalist counterparts. Our data further suggest that given the same dietary input, specialists are more competent in accumulating and thus disproportionately fortifying their fecula with water-soluble compounds. The identity and defensive characteristics of the water-soluble shield constituents await structural elucidation. Many classes of water-soluble substances, such as pyrrolizidines, steroidal alkaloids, phenolics, cardenolides, sapogenines, and flavonoids, are well known to have deterrent, repellant and/or toxic characteristics.

The Ehrlich and Raven (1964) model provided an initial framework intended to reveal evolutionary processes responsible for patterns of plant and herbivore diversity. Their model relied, in part, on the assumption that herbivorous insects have narrow host ranges because specialists can better resist or avoid the harmful physiological effects of plant compounds. Since then, other hypotheses for the evolution of dietary specialization have been proposed (Futuyma and Keese 1992; Futuyma and Moreno 1988), recognizing that an herbivore's niche is shaped by a number of interacting factors, including nutrition, distribution and abundance of hosts, competition, and tri-trophic interactions. How host-plant chemistry and natural enemies interact to influence diet evolution remains poorly understood. We conclude from our study that characteristics of these specialized insects, including both behavior and the use of host plant-derived compounds, confer the advantage of enhanced enemy-free space. Our data cannot determine whether this was the cause of the evolution of specialized diets in herbivorous insects, but they are consistent with this hypothesis.

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